

WHAT IS CLAIMED IS:

- 1 1. An isolated or recombinant nucleic acid molecule that comprises a
2 polynucleotide sequence that encodes a polypeptide selected from the group consisting of:
 - 3 a) a polypeptide having lipid A biosynthesis acyltransferase activity,
4 wherein the polypeptide comprises an amino acid sequence that is at least about 70%
5 identical to an amino acid sequence encoded by nucleotides 350-1234 (ORF 2a) of the *LOS*
6 biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
 - 7 b) a polypeptide having glycosyltransferase activity, wherein the
8 polypeptide comprises an amino acid sequence that is at least about 70% identical to an
9 amino acid sequence encoded by nucleotides 1234-2487 (ORF 3a) of the *LOS* biosynthesis
10 locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
 - 11 c) a polypeptide having glycosyltransferase activity, wherein the
12 polypeptide comprises an amino acid sequence that is at least about 50 % identical to an
13 amino acid sequence encoded by nucleotides 2786-3952 (ORF 4a) of the *LOS* biosynthesis
14 locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1 over a region at least about 100
15 amino acids in length;
 - 16 d) a polypeptide having β 1,4-GalNAc transferase activity, wherein the
17 GalNAc transferase polypeptide comprises an amino acid sequence that is at least about 77%
18 identical to an amino acid sequence as set forth in SEQ ID NO:17 over a region at least
19 about 50 amino acids in length;
 - 20 e) a polypeptide having β 1,3-galactosyltransferase activity, wherein
21 the galactosyltransferase polypeptide comprises an amino acid sequence that is at least about
22 75% identical to an amino acid sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29
23 over a region at least about 50 amino acids in length;
 - 24 f) a polypeptide having α 2,3 sialyltransferase activity, wherein the
25 sialyltransferase polypeptide comprises an amino acid sequence that is at least about 66%
26 identical over a region at least about 60 amino acids in length to an amino acid sequence as
27 set forth in one or more of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:10;

28 g) a polypeptide having sialic acid synthase activity, wherein the
29 polypeptide comprises an amino acid sequence that is at least about 70% identical to an
30 amino acid sequence encoded by nucleotides 6924-7961 of the *LOS* biosynthesis locus of *C.*
31 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

32 h) a polypeptide having sialic acid biosynthesis activity, wherein the
33 polypeptide comprises an amino acid sequence that is at least about 70% identical to an
34 amino acid sequence encoded by nucleotides 8021-9076 of the *LOS* biosynthesis locus of *C.*
35 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

36 i) a polypeptide having CMP-sialic acid synthetase activity, wherein
37 the polypeptide comprises an amino acid sequence that is at least about 65% identical to an
38 amino acid sequence encoded by nucleotides 9076-9738 of the *LOS* biosynthesis locus of *C.*
39 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

40 j) a polypeptide having acetyltransferase activity, wherein the
41 polypeptide comprises an amino acid sequence that is at least about 65% identical to an
42 amino acid sequence encoded by nucleotides 9729-10559 of the *LOS* biosynthesis locus of
43 *C. jejuni* strain OH4384 as shown in SEQ ID NO:1; and

44 k) a polypeptide having glycosyltransferase activity, wherein the
45 polypeptide comprises an amino acid sequence that is at least about 65% identical to an
46 amino acid sequence encoded by a reverse complement of nucleotides 10557-11366 of the
47 *LOS* biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1.

1 2. The isolated or recombinant nucleic acid molecule of claim 1, wherein
2 the nucleic acid comprises a polynucleotide sequence that encodes one or more polypeptides
3 selected from the group consisting of:

4 a) a sialyltransferase polypeptide that has both an α 2,3
5 sialyltransferase activity and an α 2,8 sialyltransferase activity, wherein the sialyltransferase
6 polypeptide comprises an amino acid sequence that is at least about 75% identical to an
7 amino acid sequence as set forth in SEQ ID NO:3 over a region at least about 50 amino acids
8 in length;

9 b) a GalNAc transferase polypeptide that has a β 1,4-GalNAc
10 transferase activity, wherein the GalNAc transferase polypeptide comprises an amino acid

11 sequence that is at least about 75% identical to an amino acid sequence as set forth in SEQ
12 ID NO:17 over a region at least about 50 amino acids in length; and
13 c) a galactosyltransferase polypeptide that has β 1,3-
14 galactosyltransferase activity, wherein the galactosyltransferase polypeptide comprises an
15 amino acid sequence that is at least about 75% identical to an amino acid sequence as set
16 forth in SEQ ID NO:27 over a region at least about 50 amino acids in length.

1 3. The nucleic acid molecule of claim 1, wherein the sequence
2 comparisons are performed using a BLASTP Version 2.0 algorithm with a wordlength (W)
3 of 3, G=11, E=1, and a BLOSUM62 substitution matrix.

1 4. The nucleic acid molecule of claim 1, wherein the region extends the
2 full length of the amino acid sequence of the polypeptide.

1 5. The nucleic acid molecule of claim 1, wherein:
2 a) the sialyltransferase polypeptide comprises an amino acid sequence
3 as set forth in SEQ ID NO:3, SEQ ID NO:5 SEQ ID NO:7 or SEQ ID NO:10;
4 b) the GalNAc transferase polypeptide comprises an amino acid
5 sequence as set forth in SEQ ID NO:17; and
6 c) the galactosyltransferase polypeptide comprises an amino acid
7 sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29.

1 6. The nucleic acid molecule of claim 5, wherein:
2 a) the polynucleotide sequence that encodes the sialyltransferase
3 polypeptide is at least about 75% identical to a nucleic acid sequence as set forth in SEQ ID
4 NO:2, SEQ ID NO:4, or SEQ ID NO:6 over a region at least about 50 nucleotides in length;
5 b) the polynucleotide sequence that encodes the β 1,4-GalNAc
6 transferase polypeptide is at least about 75% identical to a nucleic acid sequence as set forth
7 in SEQ ID NO:16 or over a region at least about 50 nucleotides in length; and
8 c) the polynucleotide sequence that encodes the β 1,3-
9 galactosyltransferase polypeptide is at least about 75% identical to a nucleic acid sequence

10 as set forth in SEQ ID NO:26 or SEQ ID NO:28 over a region at least about 50 nucleotides
11 in length.

1 7. The nucleic acid molecule of claim 6, wherein the sequence
2 comparisons are performed using a BLASTN Version 2.0 algorithm with a wordlength (W)
3 of 11, G=5, E=2, q= -2, and r = 1.

1 8. The nucleic acid molecule of claim 6, wherein:

2 a) the polynucleotide sequence that encodes the sialyltransferase
3 polypeptide has a nucleic acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, or
4 SEQ ID NO:6;

5 b) the polynucleotide sequence that encodes the GalNAc transferase
6 polypeptide has a nucleic acid sequence as set forth in SEQ ID NO:16; and

7 c) the polynucleotide sequence that encodes the galactosyltransferase
8 polypeptide has a nucleic acid sequence as set forth in SEQ ID NO:26 or SEQ ID NO:28.

1 9. The nucleic acid molecule of claim 5, wherein the sialyltransferase is a
2 bifunctional sialyltransferase that has both an α 2,3-sialyltransferase activity and an α 2,8-
3 sialyltransferase activity and the polynucleotide sequence that encodes the sialyltransferase
4 polypeptide is at least about 75% identical to a nucleic acid sequence as set forth in SEQ ID
5 NO:2, SEQ ID NO:4.

1 10. An expression cassette that comprises a nucleic acid molecule of claim
2 1.

1 11. An expression vector that comprises the expression cassette of claim 10.

1 12. A host cell that comprises the expression vector of claim 11.

1 13. An isolated or recombinantly produced polypeptide selected from the
2 group consisting of:

- 3 a) a polypeptide having lipid A biosynthesis acyltransferase activity,
4 wherein the polypeptide comprises an amino acid sequence that is at least about 70%
5 identical to an amino acid sequence encoded by nucleotides 350-1234 (ORF 2a) of the *LOS*
6 biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
- 7 b) a polypeptide having glycosyltransferase activity, wherein the
8 polypeptide comprises an amino acid sequence that is at least about 70% identical to an
9 amino acid sequence encoded by nucleotides 1234-2487 (ORF 3a) of the *LOS* biosynthesis
10 locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
- 11 c) a polypeptide having glycosyltransferase activity, wherein the
12 polypeptide comprises an amino acid sequence that is at least about 50 % identical to an
13 amino acid sequence encoded by nucleotides 2786-3952 (ORF 4a) of the *LOS* biosynthesis
14 locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1 over a region at least about 100
15 amino acids in length;
- 16 d) a polypeptide having β 1,4-GalNAc transferase activity, wherein the
17 GalNAc transferase polypeptide comprises an amino acid sequence that is at least about 77%
18 identical to an amino acid sequence as set forth in SEQ ID NO:17 over a region at least
19 about 50 amino acids in length;
- 20 e) a polypeptide having β 1,3-galactosyltransferase activity, wherein
21 the galactosyltransferase polypeptide comprises an amino acid sequence that is at least about
22 75% identical to an amino acid sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29
23 over a region at least about 50 amino acids in length;
- 24 f) a polypeptide having α 2,3 sialyltransferase activity, wherein the
25 sialyltransferase polypeptide comprises an amino acid sequence that is at least about 66%
26 identical to an amino acid sequence as set forth in SEQ ID NO:3, SEQ ID NO:5, SEQ ID
27 NO:7 or SEQ ID NO:10 over a region at least about 60 amino acids in length;
- 28 g) a polypeptide having sialic acid synthase activity, wherein the
29 polypeptide comprises an amino acid sequence that is at least about 70% identical to an
30 amino acid sequence encoded by nucleotides 6924-7961 of the *LOS* biosynthesis locus of *C.*
31 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

32 h) a polypeptide having sialic acid biosynthesis activity, wherein the
33 polypeptide comprises an amino acid sequence that is at least about 70% identical to an
34 amino acid sequence encoded by nucleotides 8021-9076 of the *LOS* biosynthesis locus of *C.*
35 *jejuni* strain OH4384 as shown in SEQ ID NO:1;
36 i) a polypeptide having CMP-sialic acid synthetase activity, wherein
37 the polypeptide comprises an amino acid sequence that is at least about 65% identical to an
38 amino acid sequence encoded by nucleotides 9076-9738 of the *LOS* biosynthesis locus of *C.*
39 *jejuni* strain OH4384 as shown in SEQ ID NO:1;
40 j) a polypeptide having acetyltransferase activity, wherein the
41 polypeptide comprises an amino acid sequence that is at least about 65% identical to an
42 amino acid sequence encoded by nucleotides 9729-10559 of the *LOS* biosynthesis locus of
43 *C. jejuni* strain OH4384 as shown in SEQ ID NO:1; and
44 k) a polypeptide having glycosyltransferase activity, wherein the
45 polypeptide comprises an amino acid sequence that is at least about 65% identical to an
46 amino acid sequence encoded by a reverse complement of nucleotides 10557-11366 of the
47 *LOS* biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1.

1 14. The isolated or recombinantly produced polypeptide of claim 13,
2 wherein the polypeptide is recombinantly produced and at least partially purified.

1 15. The isolated or recombinantly produced polypeptide of claim 13,
2 wherein the polypeptide is expressed by a heterologous host cell.

1 16. The isolated or recombinantly produced polypeptide of claim 15,
2 wherein the host cell is *E. coli*.

1 17. The isolated or recombinantly produced polypeptide of claim 13,
2 wherein the polypeptide is a *C. jejuni* serotype O:2 polypeptide.

1 18. The isolated or recombinantly produced polypeptide of claim 13,
2 wherein the polypeptide is a sialyltransferase polypeptide according to g) and the
3 polypeptide is selected from the group consisting of:

4 a polypeptide has both an α 2,3 sialyltransferase activity and an α 2,8
5 sialyltransferase activity and comprises an amino acid sequence that is at least 75% identical
6 to an amino acid sequence of a *cstII* sialyltransferase encoded by ORF 7a of the *LOS*
7 biosynthesis locus from *C. jejuni* strain OH4384 as set forth in SEQ ID NO:3;

8 a polypeptide that has an α 2,3 sialyltransferase activity and comprises
9 an amino acid sequence that is at least 75% identical to an amino acid sequence of a *cstII*
10 sialyltransferase from *C. jejuni* serotype O:10 as set forth in SEQ ID NO:5;

11 a polypeptide that that has an α 2,3 sialyltransferase activity and
12 comprises an amino acid sequence that is at least 75% identical to an amino acid sequence of
13 a *cstII* sialyltransferase from *C. jejuni* serotype O:41 as set forth in SEQ ID NO:7; and

14 a polypeptide that that has an α 2,3 sialyltransferase activity and
15 comprises an amino acid sequence that is at least 75% identical to an amino acid sequence of
16 a *cstII* sialyltransferase of *C. jejuni* serotype O:2 as set forth in SEQ ID NO:10.

1 19. The isolated or recombinantly produced sialyltransferase polypeptide of
2 claim 18, wherein the sialyltransferase polypeptide has an amino acid sequence selected
3 from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, and SEQ ID
4 NO:10.

1 20. The polypeptide of claim 13, wherein:

2 a) the sialyltransferase polypeptide of f) has an amino acid sequence
3 as set forth in SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:10;

4 b) the 1,4-GalNAc transferase polypeptide of d) has an amino acid
5 sequence as set forth in SEQ ID NO:17; and

6 c) the β 1,3-galactosyltransferase polypeptide of e) has an amino acid
7 sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29.

- 1 21. A reaction mixture for synthesis of a sialylated oligosaccharide, the
2 reaction mixture comprising a sialyltransferase polypeptide which has both an α 2,3
3 sialyltransferase activity and an α 2,8 sialyltransferase activity, a galactosylated acceptor
4 moiety, and a sialyl-nucleotide sugar;
5 wherein the sialyltransferase transfers a first sialic acid residue from the
6 sialyl-nucleotide sugar to the galactosylated acceptor moiety in an α 2,3 linkage, and further
7 transfers a second sialic acid residue to the first sialic acid residue in an α 2,8 linkage.
- 1 22. The reaction mixture of claim 21, wherein the sialyl-nucleotide sugar is
2 CMP-sialic acid.
- 1 23. The reaction mixture of claim 21, wherein the sialyltransferase
2 polypeptide has an amino acid sequence that is at least about 75% identical to an amino acid
3 sequence as set forth in SEQ ID NO:3 over a region at least about 50 amino acids in length.
- 1 24. The reaction mixture of claim 23, wherein the sialyltransferase
2 polypeptide has an amino acid sequence as set forth in SEQ ID NO:3.
- 1 25. The reaction mixture of claim 21, wherein the galactosylated acceptor
2 comprises a compound having the formula $\text{Gal}\beta 1,4\text{-R}$ or $\text{Gal}\beta 1,3\text{-R}$, wherein R is selected
3 from the group consisting of H, a saccharide, oligosaccharide, or an aglycone group having
4 at least one carbohydrate atom.
- 1 26. The reaction mixture of claim 21, wherein the galactosylated acceptor is
2 attached to a protein, lipid, or proteoglycan.
- 1 27. The reaction mixture of claim 21, wherein the sialylated oligosaccharide
2 is a ganglioside, a ganglioside mimic, or a carbohydrate portion of a ganglioside.

1 28. The reaction mixture of claim 21, wherein the sialylated oligosaccharide
2 is a lysoganglioside, a lysoganglioside mimic, or a carbohydrate portion of a
3 lysoganglioside.

1 29. The reaction mixture of claim 27, wherein the galactosylated acceptor
2 moiety comprises a compound having a formula selected from the group consisting of
3 Gal4Glc-R¹ and Gal3GalNAc-R²; wherein R¹ is selected from the group consisting of
4 ceramide or other glycolipid, and R² is selected from the group consisting of Gal4GlcCer,
5 (Neu5Ac3)Gal4GlcCer, and (Neu5Ac8Neu5c3)Gal4GlcCer.

1 30. The reaction mixture of claim 29, wherein the galactosylated acceptor is
2 selected from the group consisting of Gal4GlcCer, Gal3GalNAc4(Neu5Ac3)Gal4GlcCer,
3 and Gal3GalNAc4(Neu5Ac8Neu5c3)Gal4GlcCer.

1 31. The reaction mixture of claim 21, wherein the galactosylated acceptor is
2 formed by contacting an acceptor saccharide with UDP-Gal and a galactosyltransferase
3 polypeptide, wherein the galactosyltransferase polypeptide transfers the Gal residue from the
4 UDP-Gal to the acceptor.

1 32. The reaction mixture of claim 31, wherein the galactosyltransferase
2 polypeptide has β 1,3-galactosyltransferase activity and has an amino acid sequence that is at
3 least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:27 or SEQ
4 ID NO:29 over a region at least about 50 amino acids in length.

1 33. The reaction mixture of claim 32, wherein the galactosyltransferase has
2 an amino acid sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29.

1 34. The reaction mixture of claim 31, wherein the acceptor saccharide
2 comprises a terminal GalNAc residue.

1 35. The reaction mixture of claim 34, wherein the acceptor saccharide for
2 the galactosyltransferase is formed by contacting an acceptor for a GalNAc transferase with
3 UDP-GalNAc and a GalNAc transferase polypeptide, wherein the GalNAc transferase
4 polypeptide transfers the GalNAc residue from the UDP-GalNAc to the acceptor for the
5 GalNAc transferase.

1 36. The reaction mixture of claim 35, wherein the GalNAc transferase
2 polypeptide has a β 1,4-GalNAc transferase activity and has an amino acid sequence that is at
3 least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:17 over a
4 region at least about 50 amino acids in length.

1 37. The reaction mixture of claim 29, wherein the GalNAc transferase
2 polypeptide has an amino acid sequence as set forth in SEQ ID NO:17.

1 38. A method for synthesizing a sialylated oligosaccharide, the method
2 comprising incubating a reaction mixture that comprises a sialyltransferase polypeptide
3 which has both an α 2,3 sialyltransferase activity and an α 2,8 sialyltransferase activity, a
4 galactosylated acceptor moiety, and a sialyl-nucleotide sugar, under suitable conditions
5 wherein the sialyltransferase polypeptide transfers a first sialic acid residue from the sialyl-
6 nucleotide sugar to the galactosylated acceptor moiety in an α 2,3 linkage, and further
7 transfers a second sialic acid residue to the first sialic acid residue in an α 2,8 linkage.

1 39. The method of claim 38, wherein the sialylated oligosaccharide is a
2 ganglioside.

1 40. The method of claim 38, wherein the sialyltransferase polypeptide has
2 an amino acid sequence that is at least about 75% identical to an amino acid sequence as set
3 forth in SEQ ID NO:3 over a region at least about 50 amino acids in length.

1 41. The method of claim 40, wherein the sialyltransferase polypeptide has
2 an amino acid sequence as set forth in SEQ ID NO:3.

1 42. The method of claim 38, wherein the sialylated oligosaccharide is a
2 ganglioside a lysoganglioside, a ganglioside mimic, or a lysoganglioside mimic.